




PCI/GB 2004 / 0 0 0 - - -

GB04/5360 

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

I also certify that the application is now proceeding in the name as identified herein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed 

Dated 12 January 2005

BEST AVAILABLE COPY

An Executive Agency of the Department of Trade and Industry



INVESTOR IN PEOPLE

GB 0329254.7

By virtue of a direction given under Section 30 of the Patents Act 1977, the application is proceeding in the name of:

THE UNIVERSITY OF MANCHESTER,
Oxford Road,
MANCHESTER,
M13 9PL,
United Kingdom

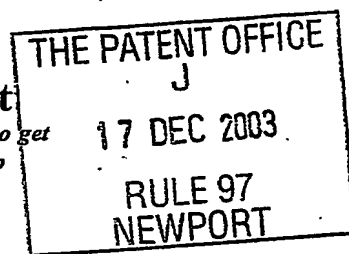
[ADP No. 07792138001]

THE Patent Office

1177
18DEC03 E660284-1/000354
E01/7700/0.00-0329254.7 NONE

Request for grant of a patent

(see the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)



The Patent Office

Cardiff Road
Newport
Gwent NP10 8QQ

1.	Your reference	AH/PN/P89533PGB		
2.	Patent application number (The Patent Office will fill in this part)	0329254.7		
3.	Full name, address and postcode of the or of each applicant (<u>underline all surnames</u>)	<p>THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY PO BOX 88, SACKVILLE STREET MANCHESTER M60 1QD</p> <p>Patents ADP number (if you know it) 773762001</p> <p>If the applicant is a corporate body, give the country/state of its incorporation UNITED KINGDOM</p>		
4.	Title of the invention	Treatment of Viral Infections		
5.	Name of your agent (if you have one)	Marks & Clerk		
	"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)	43 Park Place Leeds LS1 2RY		
	Patents ADP number (if you know it)	18013 ✓		
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number	Country	Priority application number (if you know it)	Date of filing (day/month/year)
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing (day/month/year)	
8.	Is a statement of Inventorship and of right to grant of a patent required in support of this request? (Answer: 'Yes' if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body. See note (d))	YES		

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form

Description	27 (x2)
Claim(s)	2 (x2)
Abstract	-
Drawing(s)	8 (x2)

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of Inventorship and right to grant of a patent (<i>Patents Form 7/77</i>)	-
Request for preliminary examination and search (<i>Patents Form 9/77</i>)	-
Request for substantive examination (<i>Patents Form 10/77</i>)	-
Any other documents (Please specify)	Sequence listing (14 pages)

11. I/We request the grant of a patent on the basis of this application.

Signature	<i>Marks + Clerk</i>	Date
	<u>MARKS & CLERK</u>	<u>16/12/03</u>

12. Name and daytime telephone number of person to contact in the United Kingdom
- DR A HUTTER - 0113 3895602

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.
- b) Write your answers in capital letters using black ink or you may type them.
- c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- d) If you have answered 'Yes' *Patents Form 7/77* will need to be filed.
- e) Once you have filled in the form you must remember to sign and date it.
- f) For details of the fee and ways to pay please contact the Patent Office.

Treatment of Viral Infections

The present invention relates to polypeptides, derivatives or analogues thereof, and to nucleic acids encoding the same with anti-viral activity. The invention further provides the use of such polypeptides, derivatives, analogues or nucleic acids as medicaments, and also in methods of treatment.

Antiviral agents may target one of six stages of the viral replication cycle, these being:

1. Attachment of the virus to the cell;
2. Penetration (or fusion of the viral membrane with the cell membrane);
3. Uncoating of the virus;
4. Replication of the viral nucleic acids;
5. Maturation of progeny virus particles; and
6. Release of progeny virus into extracellular fluids.

Of these six stages, replication (stage 4 above) is the target, which is most effectively influenced by conventional antiviral therapies. Attachment of the virus to the cell is however arguably a more attractive target, as the agent does not need to pass into the host cell. However, this remains an area where few successful therapies have been developed.

It is therefore one object of the present invention to provide therapeutic agents that modulate viral attachment to cells.

Lipoproteins (LPs) are globular macromolecular complexes present in serum and other extracellular fluids, consisting of lipid and protein, and are involved in the transport of lipid around the body. They have been categorised according to their density, with the main classes being high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL). Their proteins are referred to as apolipoproteins, and a number of these have been described, including apolipoproteins A, B, C, D, E, F, G, H, and J. In addition, several sub-types of apolipoproteins A, B and C have been documented.

Various interactions have been described linking LPs with viruses. These mostly involving binding of viruses to lipoproteins, with this resulting in either diminished viral infectivity, or conversely providing a 'hitchhiker' method for the virus to enter cells. Additionally, several viruses make use of cellular receptors for LPs (e.g. the LDL receptor) as a means of entering cells, although these receptors can also be released by cells as endogenous antiviral agents (for example a soluble form of the VLDL receptor is released into culture medium by HeLa cells and inhibits human rhinovirus infection). Furthermore, direct binding between certain apolipoproteins and viral proteins has also been reported. For example:

- a. Hepatitis C virus core protein binds to apolipoprotein AII;
- b. Hepatitis B virus surface antigen binds apolipoprotein H; and
- c. Simian immunodeficiency virus (SIV) gp32 protein, and human immunodeficiency virus (HIV) gp41 protein binds to apolipoprotein A1.

Work conducted in the laboratory of the inventors has shown that the presence of latent herpes simplex virus type 1 (HSV1) in brain and the possession of a particular allele of a specific gene - the APOE-e4 allele of the APOE gene - increases the risk of development of Alzheimer's disease (AD). Taken with the additional finding that APOE-e4 carriers are more likely to suffer from cold sores (which are lesions found after reactivation of HSV1 in the peripheral nervous system), these results suggested that APOE-e4 carriers are more likely to suffer damage from HSV1 infections, and suggests that there may be interactions between apolipoprotein E and certain viruses (although such interactions need not necessarily involve antiviral effects). One possible mode of interaction between HSV1 and apoE relates to the independent findings that both of these use cellular heparan sulphate proteoglycan (HSPG) molecules as their initial site of binding to cells, before subsequent attachment to secondary receptors, which raises the possibility that competition may occur at these HSPG sites between HSV1 and apoE containing LPs, which could affect viral entry.

Apolipoprotein E has been shown to have effects on the immune system (seemingly unrelated to its role in lipid metabolism) including suppression of T lymphocyte proliferation. Interactions between a number of peptides derived from residues 130-169 of apoE with lymphocytes have been examined (Clay et al.,

Biochemistry, 34: 11142-11151 (1995)). The region consisting of apoE residues 141-149 are predicted to be particularly important. Similar interactions of such peptides have been described in neuronal cell lines.

WO 94/04177 discloses that administration of particles containing lipid and amphipathic helical peptides allows clearance of toxins produced by microorganisms, and may increase the effectiveness of antibacterial drugs via an effect on bacterial membranes. However, there is no suggestion that such apoA-derived peptide containing particles may be used as antiviral medicines. It is also not clear whether administration of the peptides in particles, which is a key component of the disclosed development (whether the particles are formed before administration or endogenously), would result in effective utilisation of any antiviral action of either component of the particle.

An amphipathic helical peptide derived from apoA (described by Ananatharamiah in Meth. Enz., 128: 627-647(1986)) has been shown to prevent fusion of viral membranes with cell membranes, and furthermore prevent the fusion of membranes of infected cells (Srinivas et al. J. Cellular Biochem., 45: 224-237 (1991)). The peptide was also effective at preventing fusion for both HSV1 and HIV (Owens et al., J Clin. Invest., 86: 1142-1150 (1990)). However, the peptide had no effect at all on attachment of HSV1 at least to cells (Srinivas et al. *supra*).

Azuma et al. have reported that peptide derivatives of apoE have a strong antibacterial action, comparable with that of gentamicin (Peptides, 21: 327-330 (2000)). ApoE 133-162 was the most effective, with apoE 134-155 having little effect.

In the light of the research described above, the inventorss conducted experiments to evaluate whether or not peptides derived from ApoE (which are capable of forming helices) have antiviral activity. He found that a tandem repeat of a peptide fragment of ApoE, apoE₁₄₁₋₁₄₉ (LRKLRKRL - SEQ ID No.1), did indeed have an antiviral action. While the inventorss do not wish to bound by any hypothesis, they believe that this fragment prevents the attachment of virus particles to cells, resulting in a decrease in the infectivity of the virus as measured by a plaque

reduction assay technique. Example 1 illustrates how the peptide is effective against viruses such as HSV1, HSV2 and HIV. Accordingly, this peptide may be effective when applied to virus directly, or when applied to virus in the presence of cells, and therefore the peptide can be used to inactivate free virus particles long before they reach their target cells.

In the light of the data generated for a tandem repeat of apoE₁₄₁₋₁₄₉ (LRKLRKRL- SEQ ID No.1), the inventors decided to investigate other fragments of apolipoproteins for antiviral activity.

According to a first aspect of the present invention, there is provided a polypeptide, derivative or analogue thereof comprising a tandem repeat of apoE₁₄₁₋₁₄₉ of SEQ ID No 2 or a truncation thereof, characterised in that at least one Leucine (L) residue of SEQ ID No. 2 is replaced by a Tryptophan (W) or Tyrosine (Y) residue or derivatives thereof.

By "a tandem repeat of apoE₁₄₁₋₁₄₉ of SEQ ID No. 2" we mean the peptide with the amino acid sequence: LRKLRKRLRLRKLRKRL. This peptide is also referred to herein as GIN 1 or GIN1p (when N terminal protected by an acetyl group, and C terminal protected by an amide group).

The inventors conducted exhaustive experiments to assess the antiviral activity of peptides from apolipoproteins and derivatives thereof. Peptides and derivatives from ApoE were a particular focus. To the inventorss surprise they found that most of the peptides tested had little or no antiviral effect. The surprising exceptions were peptides according to the first aspect of the invention. Examples 2 - 4 illustrate the efficacy of the peptides according to the invention compared to a tandem repeat of apoE₁₄₁₋₁₄₉ and other peptides derived from apolipoproteins.

The inventorss have identified that tryptophan (W) or Tyrosine (Y) substituted apoE-derived peptides have antiviral activity.

The inventorss have noted that W or Y substitutions may be expected to increase the likelihood of the peptide forming an alpha helix and wondered if this may

explain the antiviral efficacy of compounds according to the first aspect of the invention. However, they do not believe this explains the surprising efficacy of peptides according to the invention. This is because a number of alternative substitutions would be expected to increase alpha helix formation (e.g see Table 1 for calculation of likelihood of various L substituted peptides forming an alpha helix). However these alternative substitutions do not result in peptides with the antiviral efficacy of peptides according to the present invention.

Table 1. *Predicted proportion of molecules of various peptides forming alpha-helix in aqueous 0.15M Na buffer at 37°C (%) (using AGADIR secondary structure prediction software available from <http://www.embl-heidelberg.de/Services/serrano/agadir/agadir-start.html>)*

Amino Acid Substitution	Sequence of peptide	% helix
E, Glu	ERKERKREEERKERKREE	6.24
A, Ala	ARKARKRAAARKARKRAA	1.85
D, Asp	DRKDRKRDDDRKDRKRDD	1.59
W, Trp	WRKWRKRWWWRKWRKRWW	1.47
M, Met	MRKMRKRMMMRKMRKRMM	1.01
Y, Tyr	YRKYRKRYYYRKYRKRY	0.8
F, Phe	FRKFRKRFFFRKFRKRFF	0.79
I, Ile	IRKIRKRIRIRKIRKRIR	0.6
Q, Gln	QRKQRKRQQQRKQRKRQQ	0.55
No swap		0.51

The inventorss have also noted:

1. The increase from the W substitution is very small (0.51% of GIN 1p molecules will form a helix, which increases marginally to 1.47% of the W substituted peptide);
2. A number of other substitutions would be predicted to increase the proportion of molecules forming an alpha helix at any one time. For instance, substituting L for E or A increases the likelihood of forming an alpha-helix beyond that of a W substitution (to 6.24 % and 1.87 % respectively). However, both of these substitutions in fact abolished antiviral activity (e.g. see peptide GIN39 in Example 3); and
3. Substituting with M would also be predicted to increase alpha helical activity, albeit to a slightly lesser degree than the W substitution (to 1.01 %).

However, the M substitution abolishes antiviral activity (see peptide 40 in Example 3).

Therefore there is no correlation between likelihood of forming an alpha helix, and the strength of antiviral activity for 'L-substituted' peptides according to the invention.

The efficacy of peptides according to the invention is all the more surprising because W (Trp) or Y (Tyr) substitution for L (Leu) will make the peptide less amphipathic. (Table 2 illustrates the accepted order of hydrophobicity of amino acids). A skilled person may actually suspect that making a peptide more amphipathic would confer antiviral character. Therefore, unexpectedly, W or Y substitutions of apoE-derived peptides result in a significant increase in their antiviral activity.

Table 2

Hydrophobicity of Amino Acids

**Phe > Leu = Ile > Tyr = Trp > Val > Met > Pro > Cys > Ala > Gly >
Thr > Ser > Lys > Gln > Asn > His > Glu > Asp > Arg**

It is preferred that the polypeptide according to the first aspect of the invention has at least two W or Y substitutions, and more preferably three or more W or Y substitutions.

The substituted polypeptide may comprise 18 amino acids (or derivatives thereof) and thereby correspond to the full length of SEQ ID No. 2. However the inventorss have surprisingly found that truncated peptides based on SEQ ID No.2 also have efficacy as antiviral agents. Accordingly preferred peptides or derivatives thereof may have less than 18 amino acids. For instance some peptides according to the first aspect of the invention may be 17, 16, 15, 14, 13, 12, 11, 10 or less amino acids in length.

It will be appreciated that modified forms of W or Y may be substituted into the tandem repeat of apoE₁₄₁₋₁₄₉. Such peptides will still have antiviral activity provided that the modification does not significantly alter its chemical characteristics.

Preferred W substituted peptides according to the first aspect of the invention have the amino acids sequence:

(a) WRKWRKRWWWRKWRKRWW (SEQ ID No. 3). This peptide corresponds to the full length tandem repeat with all Leucines substituted for tryptophan residues. This peptide is designated GIN 7 when referred to herein.

(b) WRKWRKRWRKWRKR (SEQ ID No. 4). This peptide corresponds to the full length tandem repeat with all Leucines substituted for tryptophan residues and truncated by the excision of amino acids 9, 10, 17 and 18. This peptide is designated GIN 32 when referred to herein.

(c) WRKWRKRWWLRKLRKRL (SEQ ID No. 5). This peptide corresponds to the full length tandem repeat with a subset of Leucines substituted for tryptophan residues. This peptide is designated GIN 34 when referred to herein.

A preferred Y substituted peptides according to the first aspect of the invention have the amino acids sequence YRK YRK R Y Y Y R K Y R K R Y Y (SEQ ID No. 6). This peptide corresponds to the full length tandem repeat with all Leucines substituted for tyrosine residues. This peptide is designated GIN 41 when referred to herein.

The inventors, during the course of his experiments, also established that a number of other peptides based on the ApoE tandem repeat have efficacy for treating viruses. These peptides are:

(a) LRKLRKRLLLRKLRK (SEQ ID No. 7). This peptide corresponds to a truncated form of the full length tandem repeat. This peptide has the

advantage that the peptide is shorter than GIN 1 and is therefore cheaper to manufacture. This peptide is designated GIN 4 when referred to herein.

(b) LRKLRKLRKLRKR (SEQ ID No. 8). This peptide corresponds to the full length tandem repeat truncated by the excision of amino acids 9, 10, 17 and 18. This peptide is designated GIN 8 when referred to herein.

(c) LRKLRKLRKLRKLRK (SEQ ID No. 9). This peptide corresponds to a variation of the full length tandem repeat comprising a repeat of the LRK motif. This peptide is designated GIN 9 when referred to herein.

According to a second aspect of the invention there is provided a polypeptide, derivative or analogue thereof according to the first aspect of the invention or a peptide of SEQ ID No. 7, 8 or 9 for use as a medicament.

According to a third aspect of the invention there is provided the use of a polypeptide, derivative or analogue thereof according to the first aspect of the invention or a peptide of SEQ ID No. 7, 8 or 9 for the manufacture of a medicament for treating viral infections.

It will be appreciated that the therapeutic effects of polypeptides, derivatives or analogues according to the first aspect of the invention may also be mediated "indirectly" by agents that increase the activity of such polypeptides, derivatives or analogues. The present invention provides the first medical use of such agents.

Thus, according to a fourth aspect of the invention, there is provided an agent capable of increasing the biological activity of a polypeptide, derivative or analogue according to the first aspect of the invention for use as a medicament.

Agents capable of increasing the biological activity of polypeptides, derivatives or analogues according to the invention may achieve their effect by a number of means. For instance, such agents may increase the expression of such polypeptides, derivatives or analogues. Alternatively (or in addition) such agents may

increase the half-life of polypeptides, derivatives or analogues according to the invention in a biological system, for example by decreasing turnover of the polypeptides, derivatives or analogues.

Due to their increased biological activity polypeptides, derivatives or analogues according to the first three aspects of the invention are of utility as antiviral agents.

Polypeptides, derivatives or analogues according to the first, second and third aspects of the invention may be used in the treatment of a number of viral infections. The virus may be any virus, and particularly an enveloped virus. Preferred viruses are poxviruses, iridoviruses, togaviruses, or toroviruses. A more preferred virus is a filovirus, arenavirus, bunyavirus, or a rhabdovirus. An even more preferred virus is a paramyxovirus or an orthomyxovirus. It is envisaged that virus may preferably include a hepadnavirus, coronavirus, flavivirus, or a retrovirus. Preferably, the virus includes a herpesvirus or a lentivirus. In preferred embodiments, the virus may be Human Immunodeficiency Virus (HIV), Human herpes simplex virus type 2 (HSV2), or Human herpes simplex virus type 1 (HSV1).

Polypeptides, derivatives or analogues according to the first, second and third aspects of the invention may be used to treat viral infections as a monotherapy (i.e. use of the compound alone) or in combination with other compounds or treatments used in antiviral therapy (e.g. acyclovir, gangcylovir, ribavirin, interferon, anti-HIV medicaments including nucleoside, nucleotide or non-nucleoside inhibitors of reverse transcriptase, protease inhibitors and fusion inhibitors.)

Derivatives of polypeptides according to the invention may include derivatives that increase or decrease the polypeptide's half-life *in vivo*. Examples of derivatives capable of increasing the half-life of polypeptides according to the invention include peptoid derivatives of the polypeptides, D-amino acid derivatives of the polypeptides, and peptide-peptoid hybrids.

Polypeptides according to the invention may be subject to degradation by a number of means (such as protease activity in biological systems). Such degradation

may limit the bioavailability of the polypeptides and hence the ability of the polypeptides to achieve their biological function. There are wide ranges of well-established techniques by which peptide derivatives that have enhanced stability in biological contexts can be designed and produced. Such peptide derivatives may have improved bioavailability as a result of increased resistance to protease-mediated degradation. Preferably a peptide derivative or analogue suitable for use according to the invention is more protease-resistant than the peptide from which it is derived. Protease-resistance of a peptide derivative and the peptide from which it is derived may be evaluated by means of well-known protein degradation assays. The relative values of protease resistance for the peptide derivative and peptide may then be compared.

Peptoid derivatives of the peptides of the invention may be readily designed from knowledge of the structure of the peptide according to the first aspect of the invention. Commercially available software may be used to develop peptoid derivatives according to well-established protocols.

Retropeptoids, (in which all amino acids are replaced by peptoid residues in reversed order) are also able to mimic antiviral peptides derived from apolipoproteins. A retropeptoid is expected to bind in the opposite direction in the ligand-binding groove, as compared to a peptide or peptoid-peptide hybrid containing one peptoid residue. As a result, the side chains of the peptoid residues are able point in the same direction as the side chains in the original peptide.

A further embodiment of a modified form of polypeptides according to the invention comprises D-amino acid forms of the polypeptides. In this case the order of the amino acid residues is reversed as compared to that found in SEQ ID No. 1. The preparation of peptides using D-amino acids rather than L-amino acids greatly decreases any unwanted breakdown of such an agent by normal metabolic processes, decreasing the amounts of agent which need to be administered, along with the frequency of its administration.

The polypeptides, analogues, or derivatives of the invention represent products that may advantageously be expressed by biological cells.

Thus, the present invention also provides, in a fifth aspect, a nucleic acid sequence encoding a polypeptide, derivative or analogue according to the first aspect of the invention.

Preferred nucleic acids according to the fifth aspect of the invention may include SEQ ID No.12, SEQ ID No.12, SEQ ID No.14, SEQ ID No.13, SEQ ID No.15, SEQ ID No.16, SEQ ID No.17, or SEQ ID No.18.

It will be appreciated that, due to redundancy in the genetic code, a nucleic acid sequence in accordance with the fifth aspect of the invention may vary from the naturally occurring ApoE gene providing a codon encodes a polypeptide, derivative or analogue thereof in accordance with the first aspect of the invention.

It will be appreciated that polypeptides, derivatives and analogues according to the invention represent favourable agents to be administered by techniques involving cellular expression of nucleic acid sequences encoding such molecules. Such methods of cellular expression are particularly suitable for medical use in which the therapeutic effects of the polypeptides, derivatives and analogues are required over a prolonged period.

Thus according to a sixth aspect of the present invention there is provided a nucleic acid sequence according to the fifth aspect of the invention for use as a medicament.

The nucleic acid may preferably be an isolated or purified nucleic acid sequence. The nucleic acid sequence may preferably be a DNA sequence.

The nucleic acid sequence may further comprise elements capable of controlling and/or enhancing its expression. The nucleic acid molecule may be contained within a suitable vector to form a recombinant vector. The vector may for example be a plasmid, cosmid or phage. Such recombinant vectors are highly useful in the delivery systems of the invention for transforming cells with the nucleic acid molecule.

Recombinant vectors may also include other functional elements. For instance, recombinant vectors can be designed such that the vector will autonomously replicate in the cell. In this case elements that induce nucleic acid replication may be required in the recombinant vector. Alternatively, the recombinant vector may be designed such that the vector and recombinant nucleic acid molecule integrates into the genome of a cell. In this case nucleic acid sequences, which favour targeted integration (e.g. by homologous recombination) are desirable. Recombinant vectors may also have DNA coding for genes that may be used as selectable markers in the cloning process.

The recombinant vector may also further comprise a promoter or regulator to control expression of the gene as required.

The nucleic acid molecule may (but not necessarily) be one, which becomes incorporated in the DNA of cells of the subject being treated. Undifferentiated cells may be stably transformed leading to the production of genetically modified daughter cells (in which case regulation of expression in the subject may be required e.g. with specific transcription factors or gene activators). Alternatively, the delivery system may be designed to favour unstable or transient transformation of differentiated cells in the subject being treated. When this is the case, regulation of expression may be less important because expression of the DNA molecule will stop when the transformed cells die or stop expressing the protein (ideally when the required therapeutic effect has been achieved).

The delivery system may provide the nucleic acid molecule to the subject without it being incorporated in a vector. For instance, the nucleic acid molecule may be incorporated within a liposome or virus particle. Alternatively a "naked" nucleic acid molecule may be inserted into a subject's cells by a suitable means e.g. direct endocytotic uptake.

The nucleic acid molecule may be transferred to the cells of a subject to be treated by transfection, infection, microinjection, cell fusion, protoplast fusion or ballistic bombardment. For example, transfer may be by ballistic transfection with coated gold particles, liposomes containing the nucleic acid molecule, viral vectors

(e.g. adenovirus) and means of providing direct nucleic acid uptake (e.g. endocytosis) by application of the nucleic acid molecule directly.

It will be appreciated that the polypeptides, agents, nucleic acids or derivatives according to the present invention may be used in a monotherapy (i.e. use of polypeptides, agents, nucleic acids or derivatives according to the invention alone to prevent and/or treat a viral infection). Alternatively, polypeptides, agents, nucleic acids or derivatives according to the invention may be used as an adjunct, or in combination with, known therapies.

Polypeptides, agents, nucleic acids or derivatives according to the invention may be combined in compositions having a number of different forms depending, in particular on the manner in which the composition is to be used. Thus, for example, the composition may be in the form of a powder, tablet, capsule, liquid, ointment, cream, gel, hydrogel, aerosol, spray, micelle, transdermal patch, liposome or any other suitable form that may be administered to a person or animal. It will be appreciated that the vehicle of the composition of the invention should be one which is well tolerated by the subject to whom it is given, and preferably enables delivery of the polypeptides, agents, nucleic acids or derivatives to the brain. It is preferred that the polypeptides, agents, nucleic acids or derivatives according to the invention be formulated in a manner that permits their passage across the blood brain barrier.

Compositions comprising polypeptides, agents, nucleic acids or derivatives according to the invention may be used in a number of ways. For instance, systemic administration may be required in which case the compound may be contained within a composition that may, for example, be ingested orally in the form of a tablet, capsule or liquid. Alternatively the composition may be administered by injection into the blood stream. Injections may be intravenous (bolus or infusion) or subcutaneous (bolus or infusion). The compounds may be administered by inhalation (e.g. intranasally).

Polypeptides, agents, nucleic acids or derivatives may also be incorporated within a slow or delayed release device. Such devices may, for example, be inserted on or under the skin, and the compound may be released over weeks or even months.

Such devices may be particularly advantageous when long term treatment with a polypeptide, agent, nucleic acid or derivative according to the invention is required and which would normally require frequent administration (e.g. at least daily injection).

It will be appreciated that the amount of a polypeptide, agent, nucleic acid or derivative that is required is determined by its biological activity and bioavailability which in turn depends on the mode of administration, the physicochemical properties of the polypeptide, agent, nucleic acid or derivative employed and whether the polypeptide, agent, nucleic acid or derivative is being used as a monotherapy or in a combined therapy. The frequency of administration will also be influenced by the above-mentioned factors and particularly the half-life of the polypeptide, agent, nucleic acid or derivative within the subject being treated.

Optimal dosages to be administered may be determined by those skilled in the art, and will vary with the particular polypeptide, agent, nucleic acid or derivative in use, the strength of the preparation, the mode of administration, and the advancement of the disease condition. Additional factors depending on the particular subject being treated will result in a need to adjust dosages, including subject age, weight, gender, diet, and time of administration.

Known procedures, such as those conventionally employed by the pharmaceutical industry (e.g. *in vivo* experimentation, clinical trials, etc.), may be used to establish specific formulations of polypeptides, agents, nucleic acids or derivatives according to the invention and precise therapeutic regimes (such as daily doses of the polypeptides, agents, nucleic acids or derivatives and the frequency of administration).

Generally, a daily dose of between 0.01 $\mu\text{g/kg}$ of body weight and 0.5 g/kg of body weight of polypeptides, agents, nucleic acids or derivatives according to the invention may be used for the prevention and/or treatment of a viral infection, depending upon which specific polypeptide, agent, nucleic acid or derivative is used. More preferably, the daily dose is between 0.01 mg/kg of body weight and 200 mg/kg of body weight, and most preferably, between approximately 1mg/kg and 100 mg/kg.

Daily doses may be given as a single administration (e.g. a single daily injection). Alternatively, the polypeptide, agent, nucleic acid or derivative used may require administration twice or more times during a day. As an example, polypeptides, agents, nucleic acids or derivatives according to the invention may be administered as two (or more depending upon the severity of the condition) daily doses of between 25 mg and 7000 mg (i.e. assuming a body weight of 70kg). A patient receiving treatment may take a first dose upon waking and then a second dose in the evening (if on a two dose regime) or at 3 or 4 hourly intervals thereafter. Alternatively, a slow release device may be used to provide optimal doses to a patient without the need to administer repeated doses.

This invention provides a pharmaceutical composition comprising a therapeutically effective amount of a polypeptide, agent, nucleic acid or derivative according to the invention and optionally a pharmaceutically acceptable vehicle. In one embodiment, the amount of the polypeptide, agent, nucleic acid or derivative is an amount from about 0.01 mg to about 800 mg. In another embodiment, the amount of the polypeptide, agent, nucleic acid or derivative is an amount from about 0.01 mg to about 500 mg. In another embodiment, the amount of the polypeptide, agent, nucleic acid or derivative is an amount from about 0.01 mg to about 250 mg. In another embodiment, the amount of the polypeptide, agent, nucleic acid or derivative is an amount from about 0.1 mg to about 60 mg. In another embodiment, the amount of the polypeptide, agent, nucleic acid or derivative is an amount from about 0.1 mg to about 20 mg.

This invention provides a process for making a pharmaceutical composition comprising combining a therapeutically effective amount of a polypeptide, agent, nucleic acid or derivative according to the invention and a pharmaceutically acceptable vehicle. A "therapeutically effective amount" is any amount of a polypeptide, agent, nucleic acid or derivative according to the first aspect of the invention which, when administered to a subject provides prevention and/or treatment of a viral infection. A "subject" is a vertebrate, mammal, domestic animal or human being.

A "pharmaceutically acceptable vehicle" as referred to herein is any physiological vehicle known to those of ordinary skill in the art useful in formulating pharmaceutical compositions.

In a preferred embodiment, the pharmaceutical vehicle is a liquid and the pharmaceutical composition is in the form of a solution. In another embodiment, the pharmaceutically acceptable vehicle is a solid and the composition is in the form of a powder or tablet. In a further embodiment, the pharmaceutical vehicle is a gel and the composition is in the form of a cream or the like.

A solid vehicle can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the vehicle is a finely divided solid that is in admixture with the finely divided active polypeptide, agent, nucleic acid or derivative. In tablets, the active polypeptide, agent, nucleic acid or derivative is mixed with a vehicle having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active polypeptide, agent, nucleic acid or derivative. Suitable solid vehicles include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, polyvinylpyrrolidone, low melting waxes and ion exchange resins.

Liquid vehicles are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active polypeptide, agent, nucleic acid or derivative can be dissolved or suspended in a pharmaceutically acceptable liquid vehicle such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid vehicle can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid vehicles for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their

derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the vehicle can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid vehicles are useful in sterile liquid form compositions for parenteral administration. The liquid vehicle for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by for example, intramuscular, intrathecal, epidural, intraperitoneal, intravenous and particularly subcutaneous, intracerebral or intracerebroventricular injection. The polypeptide, agent, nucleic acid or derivative may be prepared as a sterile solid composition that may be dissolved or suspended at the time of administration using sterile water, saline, or other appropriate sterile injectable medium. Vehicles are intended to include necessary and inert binders, suspending agents, lubricants, flavourants, sweeteners, preservatives, dyes, and coatings.

Polypeptides, agents, nucleic acids or derivatives according to the invention can be administered orally in the form of a sterile solution or suspension containing other solutes or suspending agents (for example, enough saline or glucose to make the solution isotonic), bile salts, acacia, gelatin, sorbitan monoleate, polysorbate 80 (oleate esters of sorbitol and its anhydrides copolymerized with ethylene oxide) and the like.

Polypeptides, agents, nucleic acids or derivatives according to the invention can also be administered orally either in liquid or solid composition form. Compositions suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.

The invention will be further described, by way of example only, with reference to the following Examples and figures in which:-

- Figure 1** shows the effect of apoE_{141-149dp} and apoE₂₆₃₋₂₈₆ on HSV1 infectivity. (points are derived from the average of up to four values) as described in Example 1;
- Figure 2** shows the effect of apoE_{141-149dp} or apoE₂₆₃₋₂₈₆ on HSV2 infectivity (points are derived from the average of up to four values) as described in Example 1;
- Figure 3** illustrates inhibition of HIV-1 p24 production, as measured by ELISA, by apoE_{141-149r}, and apoE₂₆₃₋₂₈₆ in acutely infected U937 cells (values are the average of three experiments) as described in Example 1;
- Figure 4** illustrates the effect of 4 peptides (GIN1, 1p, 2 and 3) on HSV1 infectivity as described in Example 2;
- Figure 5** illustrates the effect of 4 peptides (GIN 4-7) on HSV1 infectivity as described in Example 2;
- Figure 6** illustrates the effect of 4 peptides (GIN 8-11) on HSV1 infectivity as described in Example 2;
- Figure 7** compares and illustrates the effect of peptides GIN 7, GIN 32, GIN 34, and GIN 1p on HSV1 infectivity as described in Example 2; and
- Figure 8** illustrates the anti-HIV action of peptides GIN7 and GIN34 against HIV isolate SF162, grown in NP-2 glioma cells overexpressing CCR5 co-receptors as described in Example 4.

EXAMPLE 1

Experiments were conducted with ApoE₁₄₁₋₁₄₉ to establish whether or not the peptide had any efficacy as an antiviral agent.

1.1 HSV1

Figure 1 and table 1 show typical results for the test for anti-HSV1 activity. The assay involved treating confluent Vero cells in 24-well plates with medium containing virus and varying amounts of peptide for one hour, followed by removal of this inoculum, and addition of viscous 'overlay' medium, containing 0.2% high viscosity carboxymethylcellulose. The overlay medium only allows infection of those cells immediately adjacent to an infected cell. After 2 days incubation and then fixation and staining, small patches of infected cells (or 'plaques') are visible, which are counted. Each of these corresponds to the infection of a single cell during the one hour inoculation. ApoE_{141-149dp} produced a 40% reduction in plaque number at a concentration of around 20 μ M. Note the peptide was only present in the experimental system for 1 hour.

Table 1: HSV1 plaque formation in Vero cells after inoculation with virus containing either apoE_{141-149dp} or apoE₂₆₃₋₂₈₆. Values for untreated wells are underlined.

[μ M]	<i>ApoE_{141-149r}</i>					<i>ApoE₂₆₃₋₂₈₆</i>				
	1	2	3	4	Mean \pm sd	1	2	3	4	Mean \pm sd
<u>0</u>	<u>96</u>	<u>102</u>	<u>123</u>		<u>107 \pm 14.2</u>					
5	129	106	103	100	110 \pm 13.2	113	119	122	126	120 \pm 5.5
10	73	87	76	89	81 \pm 7.9	116	124	102		114 \pm 11.1
20	68	67	63	63	65 \pm 2.6	148	112	133	114	127 \pm 17.0
30	72	71	56		66 \pm 9.0	134	109	114	125	121 \pm 11.2
40	64	65	53	68	63 \pm 6.6	120	113	125	144	126 \pm 11.2

1.2 HSV2

Figure 2 and table 2 show typical results for the test for anti-HSV2 activity. The assay was carried out as for the anti-HSV1 assay, except Hep-2 cells were used rather than Vero cells. ApoE_{141-149dp} produced a 50% reduction in plaque number at a concentration of around 20 μ M. Again note that the peptide was only present in the experimental system for 1 hour.

Table 2. HSV2 plaque formation in HEp-2 cells after inoculation with virus containing either apoE_{141-149dp} or apoE₂₆₃₋₂₈₆. Values for untreated wells are underlined.

[μ M]	<i>ApoE</i> _{141-149dp}					<i>ApoE</i> ₂₆₃₋₂₈₆				
	1	2	3	4	Mean \pm sd	1	2	3	4	Mean \pm sd
<u>0</u>	<u>156</u>	<u>137</u>	<u>162</u>	<u>152</u>	152 \pm 10.7					
5	160	134	140	130	141 \pm 13.3	135	160	161	152	152 \pm 12.0
10	125	113	131	132	125 \pm 8.7	157	121	151	134	141 \pm 16.1
20	82	72	73	81	77 \pm 5.2	118	150	182	134	146 \pm 27.3
30	76	77	71	72	74 \pm 2.9	118	117	103	159	124 \pm 24.2
40	51	59	69	49	57 \pm 9.1	132	144	125	124	131 \pm 24.2

1.3. HIV

Figure 3 and table 3 show typical results for the test for anti-HIV activity. The assay was carried out by incubating HIV infected U937 cells in the presence of various levels of peptide for 7 days, followed by assay for levels of the HIV protein p24 in the cells using an Enzyme Linked Immunoabsorbant Assay (ELISA) technique. ApoE_{141-149dp} produced a 95% reduction in infectivity at 20 μ M. ApoE₂₆₃₋₂₈₆ produced a 20% reduction in infectivity at 20 μ M.

The effect on HIV appears at lower peptide concentrations, though this may be due to peptide being in contact with cells for 7 days, as opposed to just 1 hour in plaque reduction assays with herpes viruses.

Table 3: Inhibition of HIV-1 p24 production, as measured by ELISA, by apoE_{141-149dp}, and apoE₂₆₃₋₂₈₆ in acutely infected U937 cells.

[μM]	% Decrease in HIV p24 Production							
	ApoE _{141-149dp}				ApoE ₂₆₃₋₂₈₆			
	Exp.1	Exp.2	Exp.3	Mean ± sd	Exp.1	Exp.2	Exp.3	Mean ± sd
0	0	0	0	0	0	0	0	0
10	91.66	70.31	89.85	83.94 ± 11.84	31.75	8.50	29.38	23.21 ± 12.79
20	96.87	95.08	93.10	95.02 ± 1.89	7.69	29.71	30.91	22.77 ± 13.07
30	95.94	88.63	87.77	90.78 ± 4.49	37.94	27.83	41.78	35.85 ± 7.21
40	96.80	95.47	95.33	95.87 ± 0.81	23.50	30.08	48.04	38.87 ± 12.70
50	95.73	93.25	95.38	94.79 ± 1.34	33.36	41.45	45.66	40.16 ± 6.25

The results presented in 1.1 – 1.3 illustrate that ApoE_{141-149dp} was more efficacious than ApoE₂₆₃₋₂₈₆.

In the light of these results, the inventors proceeded to test other peptides generated from apolipoproteins to investigate whether or not such peptides had antiviral activity (see Example 2).

EXAMPLE 2

Given the knowledge gained by the inventors following the work reported in Example 1, experiments were conducted to evaluate the antiviral effects of a large number of peptides derived from apolipoproteins. Surprisingly, the inventors found that only a minority of the peptides tested had antiviral effects (see 2.2). Such peptides represent peptides according to the invention.

2.1 Materials and Methods

2.1.1 Cell culture.

African Green Monkey Kidney (Vero) cells were maintained in Eagle's minimum essential medium with Earle's salt (EMEM) and supplemented with 10% fetal calf serum (heat-inactivated), 4 mM L-glutamine, and 1% (v/v) nonessential amino acids, plus penicillin and streptomycin (100 IU/mg and 100 mg/ml, respectively) (maintenance medium referred to as 10% EMEM). The cells were incubated at 37°C in a humidified atmosphere of air with 5% CO₂.

On harvesting, monolayers were washed in phosphate-buffered saline (PBS), and dislodged by incubating with trypsin in PBS for 30 min, before inactivating trypsin by addition of an equal volume of 10% EMEM and centrifuging at 500g (5 min, 4°C). Cell pellets were resuspended in 10% EMEM, prior to cell counting and seeding of 24-well plates. For antiviral assays, medium containing only 0.5% FCS was used (referred to as 0.5% EMEM).

2.1.2 Virus

Three separate passages of HSV1 virus were prepared by infecting Vero cells, and preparing semi-pure suspensions of virus from tissue culture supernatant and cell lysates, before freezing aliquots of virus at -85°C. Viral infectivity was assessed by carrying out plaque assays on serial dilutions of thawed aliquots (expressed in pfu/ml).

2.1.3 Peptides

Peptides were obtained in lyophilised form from a commercial supplier (AltaBioscience, University of Birmingham), and were produced at 5 micromole scale. N-terminals were protected by addition of an acetyl group, and the C-terminals were protected by addition of an amide group.

Small quantities of peptide were weighed in sterile Eppendorf tubes, before addition of sufficient 0.5% EMEM to produce a 1.5 mM stock solution, which was frozen at -20°C in aliquots.

2.1.4 Plaque reduction assays.

Vero cells were seeded at 125,000 cells per well in 10% EMEM, and were incubated overnight resulting in confluent monolayers. Peptides were diluted in 0.5% EMEM to give 2x final desired concentration, and 100 μ l aliquots were arranged on 96-well plates in arrangement to be used for 24-well plate; control wells containing normal 0.5% EMEM were also prepared. Virus stocks (p3) were thawed, and diluted in 0.5% EMEM such that there were around 100 pfu in 100 μ l. Each 24-well plate was inoculated separately. Firstly 100 μ l of virus stock was added to the peptide or control medium arranged on a 96-well plate. This was incubated at 37°C for ten minutes before inoculation. Medium was removed from four wells of a 24-well plate containing confluent Vero, and the 200 μ l inoculum added to the appropriate well. Once all wells were treated, the 24-well plate was incubated for a further 60-80 minutes. Finally the peptide-containing inoculum was removed, and 1ml of 1%EMEM containing 1% carboxymethylcellulose was added to each well. Plates were incubated for a further 22 hours, before removal of overlay, and addition of 10% formaldehyde in PBS. After a further one hour incubation, fixative was removed, monolayers washed several times with tap water, and stained with carbol fuchsin solubilised in water. After 30 minutes stain was removed, and plates washed several times with tap water, before being air dried. Plaques were counted using an Olympus IX70 Inverting Microscope, and antiviral effect expressed as a percentage of the control value for each peptide concentration. The IC₅₀ was calculated from plots of inhibitory effect against peptide concentration.

2.1.5 Toxicity Testing.

Vero cells were seeded in 96-well plates at 30,000 cells per well in 10% EMEM, and were incubated overnight resulting in confluent monolayers. GIN peptides were diluted in 0.5% EMEM to give final desired concentration, and 100 μ l aliquots were arranged on separate non-cell containing 96-well plates, prior to taking Vero 96-well plates, removing 10%EMEM, and adding 0.5% EMEM containing peptides. After incubating for 48 hours, 25 μ l of 1.5mg/ml MTT solution (in 0.5% EMEM) was added per well, and plates returned to incubator for one hour. Finally, medium was removed from wells, and blue formazan crystals solubilised by addition of 100 μ l of dimethylsulphoxide (DMSO). Absorbance of resulting solutions was then measured at 570 nm, and toxic effect expressed as a percentage of the control value for each

peptide concentration. Where possible, the EC₅₀ was calculated from plots of toxic effect against peptide concentration. Fortunately, no evidence of toxicity was found for the cell line tested, using peptide at 40 μ M exposed to cells for 2 days.

2.2 Results

Table 4 summarises data obtained for 16 peptides identified as GIN 1, GIN 1p and GIN 2 – 15.

Table 4

Peptide	Apparent IC ₅₀ (μ M)	Sequence	Size
GIN 1	16.5	LRKLRKRLLLRKLRKRL	18
GIN 1p	10	LRKLRKRLLLRKLRKRL	18
GIN 2	>40	LRKRLLLRKLRKRL	15
GIN 3	No Activity	RLLRKLRKRL	12
GIN 4	29.5	LRKLRKRLLLRKLRK	15
GIN 5	>40	LRKLRKRLLLRK	12
GIN 6	>40	ERKERKREEERKERKREE	18
GIN 7	<5	WRKWRKRWWWRKWRKRWW	18
GIN 8	13	LRKLRKRLRKLRKR	14
GIN 9	15.5	LRKLRKLRKLRKLRKLRK	18
GIN 10	39	RLLRLLRLLRLLRLLRLL	18
GIN 11	36.5	QSTEELRVRLASHLRKLRKRL	22
GIN 12	>40	LRKLRKRLLR DADDLQKRLA	20
GIN 13	>40	RDADDLQKR RDADDLQKR	20
GIN 14	>40	GERLRARMEGERLRARME	18
GIN 15	>40	RLRARMEEMRLRARMEEM	18

Figure 4 illustrates that the ApoE_{141-149dp} (labelled as GIN 1) had good efficacy for reducing HSV1 infectivity. A related peptide GIN 1p (GIN 1 with N and C terminal protection) had similar efficacy.

As illustrated in Table 4 the inventors tested a number of other related peptides (identified as GIN 2, GIN 3, GIN 4, GIN 5, GIN 6, GIN 10, GIN 11, GIN 12, GIN 13, GIN 14 and GIN 15) and it was found that they had no, or poor, efficacy for reducing viral infectivity.

In addition, the inventors found to his surprise, that a subset of the tested peptides (which are peptides according to the present invention) were effective as antiviral agents. **Figure**

5 illustrates that the peptide designated GIN 7 had efficacy for reducing HSV-1 infectivity.

Figure 6 illustrates that the peptides designated GIN 8 and GIN 9 also had efficacy for reducing HSV-1 infectivity.

Table 5 and Figure 4a illustrate that a number of peptides related or similar to the ApoE_{141-149dp} peptide (identified as peptides GIN 17 – 31 in Table 4a) had no, or poor, efficacy for reducing viral infectivity. The inventorss had rationally designed these molecules in the expectation that they may have anti-HSV1 activity and, based on the data presented in Table 4, a skilled person may have expected such peptides to have similar efficacy to those claimed according to the invention. The fact that these peptides had little effect makes the usefulness of the claimed peptides all the more surprising.

Table 5

Peptide	Apparent IC ₅₀ (μ M)	Sequence	Size
GIN 17	NA	RALVDTLKFVTQAEAGAK	17
GIN 18	NA	PYLDDFQKKWQEEMELYRQKVE	22
GIN 19	NA	PLGEEMRDRARAHVDAIRTHLA	22
GIN 20	NA	PYSDELRQRLAARLEALKENG	22
GIN 21	NA	ARLAEYHAKATEHLSTLSEKAK	22
GIN 22	36	DWLKAFYDKVAEKLKEAF	18
GIN 23	NA	PVLDEFREKLNEELEALKQKMK	22
GIN 24	NA	VTDYGKDLMEKVKSPQL	18
GIN 25	NA	VTDYGKDLMEKVKEWLNS	18
GIN 26	NA	NFHAMFQPFLEMIHEAQQ	28
GIN 27	NA	CKNKEKKCCKNKEKKC	18
GIN 28	NA	LRKEKKRLLLRKEKKRLL	18
GIN 29	38.5	HMLDVMQDHFSSRASSIDEL	20
GIN 30	NA	LQVAERLTRKYNELLKSYQ	19
GIN 31	NA	KFMETVAEKALQEQYRK	16

EXAMPLE 3

A further set of experiments were conducted on expanded number of peptides to further evaluate the effect of peptides according to the invention against HSV-1.

Table 6 confirms that the peptides designated GIN 1p and GIN 7 had anti-HSV-1 properties, whereas the peptides designated GIN 32, 34 and 41 also had efficacy.

Figure 7 compares and illustrates the effect of peptides GIN 7, GIN 32, GIN 34, and GIN 1p on HSV1 infectivity.

EXAMPLE 4

Similar experiments to those described in Example 2 were conducted to test the efficacy of the peptides according to the invention against HIV infection. The glioma cell line NP2 over-expressing both CD4 and the appropriate co-receptor (CCR5 or CXCR4) were maintained in DMEM supplemented with 10% FCS. 2×10^4 cells were plated per well of a 48-well plate 24h prior to infection and grown at 37C. The cells were then washed, and incubated in DMEM/FCS containing peptide concentrations ranging from 0.1 to 10 micromolar, at 37C for 30 minutes. 200 focus-forming units of HIV-1 stocks were then added to each well, and the cells incubated at 37C for a further 2 hours. The cells were then washed twice in PBS and fresh medium replaced. After 3 day's growth the cells were fixed in cold methanol:acetone, and stained in situ for expression of HIV-1 p24 using a monoclonal anti-p24 followed by a secondary anti-mouse beta-galactosidase conjugate. Expression was visualised by X-Gal staining and infectious foci enumerated by light-microscopy.

It was found that peptides according to the invention had similar efficacy against HSV-1 and HIV.

Figure 8 illustrates the anti-HIV action of peptides 7 and 34 against HIV isolate SF162, grown in NP-2 glioma cells overexpressing CCR5 co-receptors.

Similar data was generated for other HIV strains, and in other host cells types. Notably GIN 1p (apoEdp) had no detectable anti-HIV activity in the one combination of HIV strain and cell type against which this peptide was tested, and at the concentrations used here (up to $10\mu\text{M}$). This would suggest the W substituted peptides according to the present invention are more potent against HIV than GIN 1p (apoEdp).

Table 6. Activity of apoE-derived peptides against Herpes Simplex Virus type 1.

Peptide GIN Reference	Peptide SEQ ID No.	Nucleic acid SEQ ID No.	Sequence	IC50 (μ M)
7	SEQ ID No.3	SEQ ID No.12	WRKWRKRWWWRKWRKRWW	3.5
34	SEQ ID No.5	SEQ ID No.14	WRKWRKRWWLRKLRKRL	6
32	SEQ ID No.4	SEQ ID No.13	WRKWRKRWRKWRKR	10
41	SEQ ID No.6	SEQ ID No.15	YRKYRKRYYYRKYRKRY	16
1p	SEQ ID No.2	SEQ ID No.11	LRKLRKRLLRKLRKRL	17
Sequences where activity low:				
4	SEQ ID No.7	SEQ ID No.16	LRKLRKRLLRKLRK	29.5
22	SEQ ID No.19	NA	DWLKAFYDKVAEKLKEAF	36
11	SEQ ID No.20	NA	QSTBELRVRLASHLRKLRKRL	36.5
29	SEQ ID No.21	NA	HMLDVMQDHFSSASSIDEL	38.5
10	SEQ ID No.22	NA	RLLRLRLRLRLRLRL	39
44	SEQ ID No.23	NA	LRQLRQRLRLRLRQLRQL	40
2	SEQ ID No.24	NA	LRKRLRLRLRLRLRL	>40
5	SEQ ID No.25	NA	LRKLRKRLLRK	>40
6	SEQ ID No.26	NA	ERKERKRBEERKERKREE	>40
12	SEQ ID No.27	NA	LRKLRKRLLR DADDLQKRLA	>40
13	SEQ ID No.28	NA	RDADDLQKR RDADDLQKR	>40
14	SEQ ID No.29	NA	GERLRARMEGERLRARME	>40
15	SEQ ID No.30	NA	RLRARMEEMRLRARMEEM	>40
Sequences where activity not detectable:				
apoE 141-149	SEQ ID No.1	SEQ ID No.10	LRKLRKRL	NA
3	SEQ ID No.31	NA	RLLLRKLRKRL	NA
6	SEQ ID No.32	NA	ERKERKRBEERKERKREE	NA
17	SEQ ID No.33	NA	RALVDTLKFVTQAEGAK	NA
18	SEQ ID No.34	NA	PYLDDFQKKWQEEMLYRQKVE	NA
19	SEQ ID No.35	NA	PLGEEMRDRARAHVDALRTHLA	NA
20	SEQ ID No.36	NA	PYSDELQRLLAARLEALKENG	NA
21	SEQ ID No.37	NA	ARLAEYHAKATEHLSTLSEKAK	NA
23	SEQ ID No.38	NA	PVLDEFREKLNEELEALKQKMK	NA
24	SEQ ID No.39	NA	VTDYGKDLMEKVKSPQLQ	NA
25	SEQ ID No.40	NA	VTDYGKDLMEKVKEWLNS	NA
26	SEQ ID No.41	NA	NFHAMFQPFLEMIHEAQQ	NA
27	SEQ ID No.42	NA	CKNKEKKCKCKNKEKKC	NA
28	SEQ ID No.43	NA	LRKEKKRLLLRKEKKRL	NA
30	SEQ ID No.44	NA	LQVAERLTRKYNELLKSYQ	NA
31	SEQ ID No.45	NA	KFMETVAEKALQEYRK	NA
39	SEQ ID No.46	NA	ARKARKRAAARKARKRAA	NA
40	SEQ ID No.47	NA	MRKMRKRMMMRKMRKRMM	NA
42	SEQ ID No.48	NA	LRWLRWRLLLRWLRWRLL	NA
45	SEQ ID No.49	NA	LWKLWKWLLLWKLWKWLL	NA
46	SEQ ID No.50	NA	LYKLYKYLLLYKLYKYLL	NA
47	SEQ ID No.51	NA	LQKLQKQLLLQKLQKQLL	NA

CLAIMS

1. A polypeptide, derivative or analogue thereof comprising a tandem repeat of apoE₁₄₁₋₁₄₉ of SEQ ID No 2 or a truncation thereof characterised in that at least one Leucine (L) residue of SEQ ID No. 2 is replaced by a Tryptophan (W) residue or a derivative thereof.
2. The polypeptide, derivative or analogue thereof according to claim 1 with the amino acid sequence: WRKWRKRWWWRKWRKRWW (SEQ ID No. 3).
3. The polypeptide, derivative or analogue thereof according to claim 1 with the amino acid sequence: WRKWRKRWRKWRKR (SEQ ID No. 4).
5. The polypeptide, derivative or analogue thereof according to claim 1 with the amino acid sequence: WRKWRKRWWLRKLRKRL (SEQ ID No. 5).
6. The polypeptide, derivative or analogue thereof according to claim 1 with the amino acid sequence: YRK YRK RYYRK YRK RYY (SEQ ID No. 6).
7. The polypeptide, derivative or analogue according to any preceding claim which is a peptoid analogue.
8. The polypeptide, derivative or analogue according to any preceding claim which is a peptide/peptoid hybrid.
9. A polypeptide, derivative or analogue thereof comprising LRKLRKRLLRKLRK (SEQ ID No. 7).
10. A polypeptide, derivative or analogue thereof comprising LRKLRKRLRKLRLR (SEQ ID No. 8).
11. A polypeptide, derivative or analogue thereof comprising LRKLRKLRKLRKLRKLRK (SEQ ID No. 9).

12. A polypeptide, derivative or analogue according to any preceding claim for use as a medicament.

13. The use of an agent capable of increasing the biological activity of a polypeptide, derivative or analogue according to any preceding claim in the manufacture of a medicament for treating viral infections.

14. A method of preventing and/or treating a viral infection, comprising administering to a subject in need of such treatment a therapeutically effective amount of a polypeptide, derivative or analogue according to any preceding claim.

15. A nucleic acid sequence encoding a polypeptide, derivative or analogue according to any of claims 2 to 5 or 9 to 11.

16. A nucleic acid sequence according to claim 15 for use as a medicament.

17. A method of preventing and/or treating a viral infection comprising administering to a subject in need of such treatment a therapeutically effective amount of a nucleic acid sequence according to claim 15 or 16.

ApoE sequence.ST25
SEQUENCE LISTING

<110> University of Manchester Institute of Science and Technology (UMIST)

<120> Treatment of Viral Infections

<130> P560156

<160> 51

<170> PatentIn version 3.1

<210> 1

<211> 9

<212> PRT

<213> Homo sapiens

<400> 1

Leu Arg Lys Leu Arg Lys Arg Leu Leu
1 5

<210> 2

<211> 18

<212> PRT

<213> Homo sapiens

<400> 2

Leu Arg Lys Leu Arg Lys Arg Leu Leu Leu Arg Lys Leu Arg Lys Arg
1 5 10 15

Leu Leu

<210> 3

<211> 18

<212> PRT

<213> Homo sapiens

ApoE sequence.ST25

<400> 3

Trp Arg Lys Trp Arg Lys Arg Trp Trp Trp Arg Lys Trp Arg Lys Arg
1 5 10 15

Trp Trp

<210> 4

<211> 14

<212> PRT

<213> Homo sapiens

<400> 4

Trp Arg Lys Trp Arg Lys Arg Trp Arg Lys Trp Arg Lys Arg
1 5 10

<210> 5

<211> 18

<212> PRT

<213> Homo sapiens

<400> 5

Trp Arg Lys Trp Arg Lys Arg Trp Trp Leu Arg Lys Leu Arg Lys Arg
1 5 10 15

Leu Leu

<210> 6

<211> 18

<212> PRT

<213> Homo sapiens

<400> 6

Tyr Arg Lys Tyr Arg Lys Arg Tyr Tyr Tyr Arg Lys Tyr Arg Lys Arg
1 5 10 15

Tyr Tyr

ApoE sequence.ST25

<210> 7

<211> 15

<212> PRT

<213> Homo sapiens

<400> 7

Leu Arg Lys Leu Arg Lys Arg Leu Leu Leu Arg Lys Leu Arg Lys
1 5 10 15

<210> 8

<211> 14

<212> PRT

<213> Homo sapiens

<400> 8

Leu Arg Lys Leu Arg Lys Arg Leu Arg Lys Leu Arg Lys Arg
1 5 10

<210> 9

<211> 18

<212> PRT

<213> Homo sapiens

<400> 9

Leu Arg Lys Leu Arg Lys Leu Arg Lys Leu Arg Lys Leu Arg Lys Leu
1 5 10 15

Arg Lys

<210> 10

<211> 27

<212> DNA

<213> Homo sapiens

<400> 10

cttcgtaaac ttcgtaaacg tcttctt

<210> 11

ApoE sequence.ST25

<212> 54

<213> DNA

<213> Homo sapiens

<400> 11
cttcgtaaac ttcgtaaacg tcttcttctt cgtaaacttc gtaaacgtct tctt

54

<210> 12

<211> 54

<212> DNA

<213> Homo sapiens

<400> 12
tggcgtaaata ggcgtaaacg ttggtgggtgg cgtaaattggc gtaaacgttg gtgg

54

<210> 13

<211> 42

<212> DNA

<213> Homo sapiens

<400> 13
tggcgtaaata ggcgtaaacg ttggcgtaaa tggcgtaaac gt

42

<210> 14

<211> 54

<212> DNA

<213> Homo sapiens

<400> 14
tggcgtaaata ggcgtaaacg ttggtggctt cgtaaacttc gtaaacgtct tctt

54

<210> 15

<211> 54

<212> DNA

<213> Homo sapiens

<400> 15
tatcgtaaata atcgtaaacg ttattattat cgtaaataatc gtaaacgtta ttat

54

ApoE sequence.ST25

<210> 16

<211> 45

<212> DNA

<213> Homo sapiens

<400> 16

cttcgtaaag ttcgtaaag tcttcttctt cgtaaacttc gtaaa 45

<210> 17

<211> 42

<212> DNA

<213> Homo sapiens

<400> 17

cttcgtaaag ttcgtaaag tcttcgtaaa cttcgtaaag gt 42

<210> 18

<211> 54

<212> DNA

<213> Homo sapiens

<400> 18

cttcgtaaag ttcgtaaact tcgtaaactt cgtaaacttc gtaaacttcg taaa 54

<210> 19

<211> 18

<212> PRT

<213> Homo sapiens

<400> 19

Asp Trp Leu Lys Ala Phe Tyr Asp Lys Val Ala Glu Lys Leu Lys Glu
1 5 10 15

Ala Phe

<210> 20

<211> 22

<212> PRT

ApoE sequence.ST25

<210> Homo sapiens

<400> 20

Gln Ser Thr Glu Glu Leu Arg Val Arg Leu Ala Ser His Leu Arg Lys
1 5 10 15

Leu Arg Lys Arg Leu Leu
20

<210> 21

<211> 20

<212> PRT

<213> Homo sapiens

<400> 21

His Met Leu Asp Val Met Gln Asp His Phe Ser Arg Ala Ser Ser Ile
1 5 10 15

Ile Asp Glu Leu
20

<210> 22

<211> 18

<212> PRT

<213> Homo sapiens

<400> 22

Arg Leu Leu Arg Leu Leu Arg Leu Leu Arg Leu Leu Arg
1 5 10 15

Leu Leu

<210> 23

<211> 18

<212> PRT

<213> Homo sapiens

<400> 23

Leu Arg Gln Leu Arg Gln Arg Leu Leu Leu Arg Gln Leu Arg Gln Arg
1 5 10 15

ApoE sequence.ST25

Leu Leu

<210> 24

<211> 15

<212> PRT

<213> Homo sapiens

<400> 24

Leu Arg Lys Arg Leu Leu Leu Arg Lys Leu Arg Lys Arg Leu Leu
1 5 10 15

<210> 25

<211> 12

<212> PRT

<213> Homo sapiens

<400> 25

Leu Arg Lys Leu Arg Lys Arg Leu Leu Leu Arg Lys
1 5 10

<210> 26

<211> 18

<212> PRT

<213> Homo sapiens

<400> 26

Glu Arg Lys Glu Arg Lys Arg Glu Glu Glu Arg Lys Glu Arg Lys Arg
1 5 10 15

Glu Glu

<210> 27

<211> 20

<212> PRT

<213> Homo sapiens

ApoE sequence.ST25

<400> 27

Leu Arg Lys Leu Arg Lys Arg Leu Leu Arg Asp Ala Asp Asp Leu Gln
1 5 10 15

Lys Arg Leu Ala
20

<210> 28

<211> 18

<212> PRT

<213> Homo sapiens

<400> 28

Arg Asp Ala Asp Asp Leu Gln Lys Arg Arg Asp Ala Asp Asp Leu Gln
1 5 10 15

Lys Arg

<210> 29

<211> 18

<212> PRT

<213> Homo sapiens

<400> 29

Gly Glu Arg Leu Arg Ala Arg Met Glu Gly Glu Arg Leu Arg Ala Arg
1 5 10 15

Met Glu

<210> 30

<211> 18

<212> PRT

<213> Homo sapiens

<400> 30

Arg Leu Arg Ala Arg Met Glu Glu Met Arg Leu Arg Ala Arg Met Glu
1 5 10 15

Glu Met

ApoE sequence.ST25

<210> 31

<211> 12

<212> PRT

<213> Homo sapiens

<400> 31

Arg Leu Leu Leu Arg Lys Leu Arg Lys Arg Leu Leu
1 5 10

<210> 32

<211> 18

<212> PRT

<213> Homo sapiens

<400> 32

Glu Arg Lys Glu Arg Lys Arg Glu Glu Glu Arg Lys Glu Arg Lys Arg
1 5 10 15

Glu Glu

<210> 33

<211> 17

<212> PRT

<213> Homo sapiens

<400> 33

Arg Ala Leu Val Asp Thr Leu Lys Phe Val Thr Gln Ala Glu Gly Ala
1 5 10 15

Lys

<210> 34

<211> 22

<212> PRT

<213> Homo sapiens

ApoE sequence.ST25

<210> 34

Pro Tyr Leu Asp Asp Phe Gln Lys Lys Trp Gln Glu Glu Met Glu Leu
1 5 10 15

Tyr Arg Gln Lys Val Glu
20

<210> 35

<211> 22

<212> PRT

<213> Homo sapiens

<400> 35

Pro Leu Gly Glu Glu Met Arg Asp Arg Ala Arg Ala His Val Asp Ala
1 5 10 15

Leu Arg Thr His Leu Ala
20

<210> 36

<211> 22

<212> PRT

<213> Homo sapiens

<400> 36

Pro Tyr Ser Asp Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala
1 5 10 15

Leu Lys Glu Asn Gly Gly
20

<210> 37

<211> 22

<212> PRT

<213> Homo sapiens

<400> 37

Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu Ser Thr
1 5 10 15

Leu Ser Glu Lys Ala Lys
20

ApoE sequence.ST25

<210> 38
 <211> 22
 <212> PRT
 <213> Homo sapiens

<400> 38
 Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu Glu Leu Glu Ala
 1 5 10 15
 Leu Lys Gln Lys Met Lys
 20

<210> 39
 <211> 18
 <212> PRT
 <213> Homo sapiens

<400> 39
 Val Thr Asp Tyr Gly Lys Asp Leu Met Glu Lys Val Lys Ser Pro Glu
 1 5 10 15
 Leu Gln

<210> 40
 <211> 18
 <212> PRT
 <213> Homo sapiens

<400> 40
 Val Thr Asp Tyr Gly Lys Asp Leu Met Glu Lys Val Lys Glu Trp Leu
 1 5 10 15
 Asn Ser

<210> 41
 <211> 18
 <212> PRT

ApoE sequence.ST25

<400> Homo sapiens

<400> 41

Asn Phe His Ala Met Phe Gln Pro Phe Leu Glu Met Ile His Glu Ala
1 5 10 15

Gln Gln

<210> 42

<211> 16

<212> PRT

<213> Homo sapiens

<400> 42

Cys Lys Asn Lys Glu Lys Lys Cys Cys Lys Asn Lys Glu Lys Lys Cys
1 5 10 15

<210> 43

<211> 18

<212> PRT

<213> Homo sapiens

<400> 43

Leu Arg Lys Glu Lys Lys Arg Leu Leu Leu Arg Lys Glu Lys Lys Arg
1 5 10 15

Leu Leu

<210> 44

<211> 19

<212> PRT

<213> Homo sapiens

<400> 44

Leu Gln Val Ala Glu Arg Leu Thr Arg Lys Tyr Asn Glu Leu Lys
1 5 10 15

Ser Tyr Gln

ApoE sequence.ST25

<210> 45

<211> 16

<212> PRT

<213> Homo sapiens

<400> 45

Lys Phe Met Glu Thr Val Ala Glu Lys Ala Leu Gln Glu Tyr Arg Lys
1 5 10 15

<210> 46

<211> 18

<212> PRT

<213> Homo sapiens

<400> 46

Ala Arg Lys Ala Arg Lys Arg Ala Ala Ala Arg Lys Ala Arg Lys Arg
1 5 10 15

Ala Ala

<210> 47

<211> 18

<212> PRT

<213> Homo sapiens

<400> 47

Met Arg Lys Met Arg Lys Arg Met Met Met Arg Lys Met Arg Lys Arg
1 5 10 15

Met Met

<210> 48

<211> 18

<212> PRT

<213> Homo sapiens

ApoE sequence.ST25

<400> 48

Leu Arg Trp Leu Arg Trp Arg Leu Leu Leu Arg Trp Leu Arg Trp Arg
1 5 10 15

Leu Leu

<210> 49

<211> 18

<212> PRT

<213> Homo sapiens

<400> 49

Leu Trp Lys Leu Trp Lys Trp Leu Leu Leu Trp Lys Leu Trp Lys Trp
1 5 10 15

Leu Leu

<210> 50

<211> 18

<212> PRT

<213> Homo sapiens

<400> 50

Leu Tyr Lys Leu Tyr Lys Tyr Leu Leu Leu Tyr Lys Leu Tyr Lys Tyr
1 5 10 15

Leu Leu

<210> 51

<211> 18

<212> PRT

<213> Homo sapiens

<400> 51

Leu Gln Lys Leu Gln Lys Gln Leu Leu Leu Gln Lys Leu Gln Lys Gln
1 5 10 15

Leu Leu

FIG. 1

Reduction in HSV1 infectivity after treatment with various concentrations of apoE derived peptides

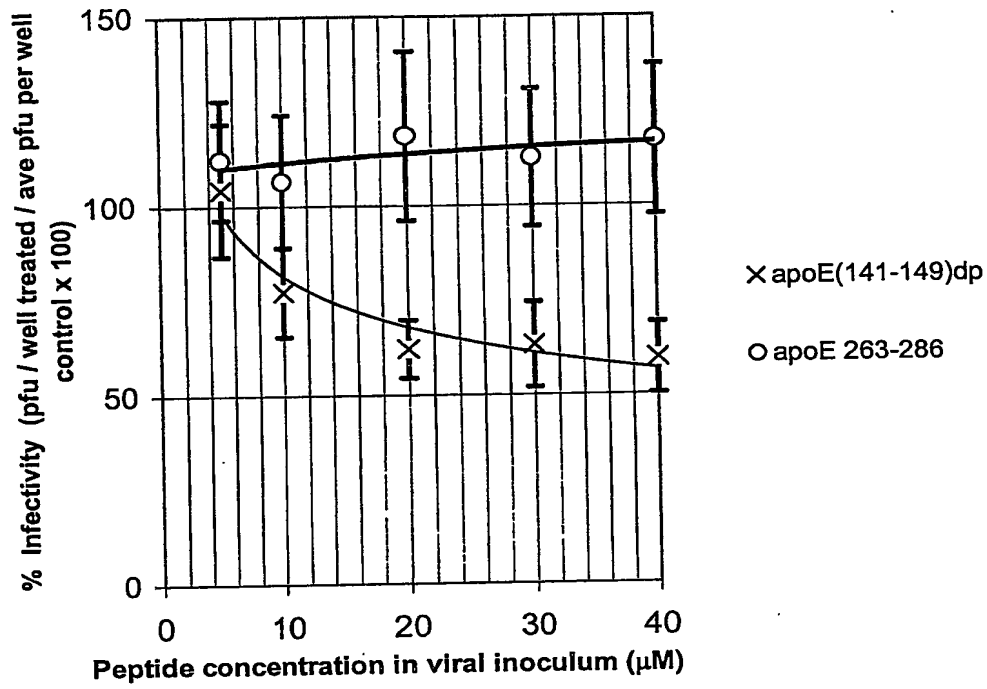


FIG. 2

Reduction in HSV2 infectivity after treatment with various concentrations of apoE derived peptides

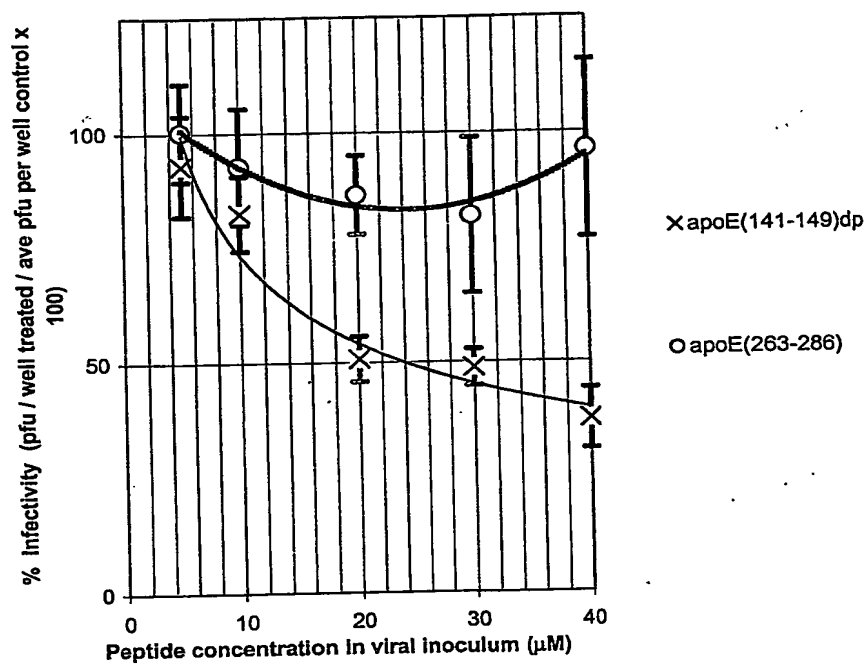


FIG. 3

Reduction in HIV infectivity after treatment with various concentrations of apoE derived peptides

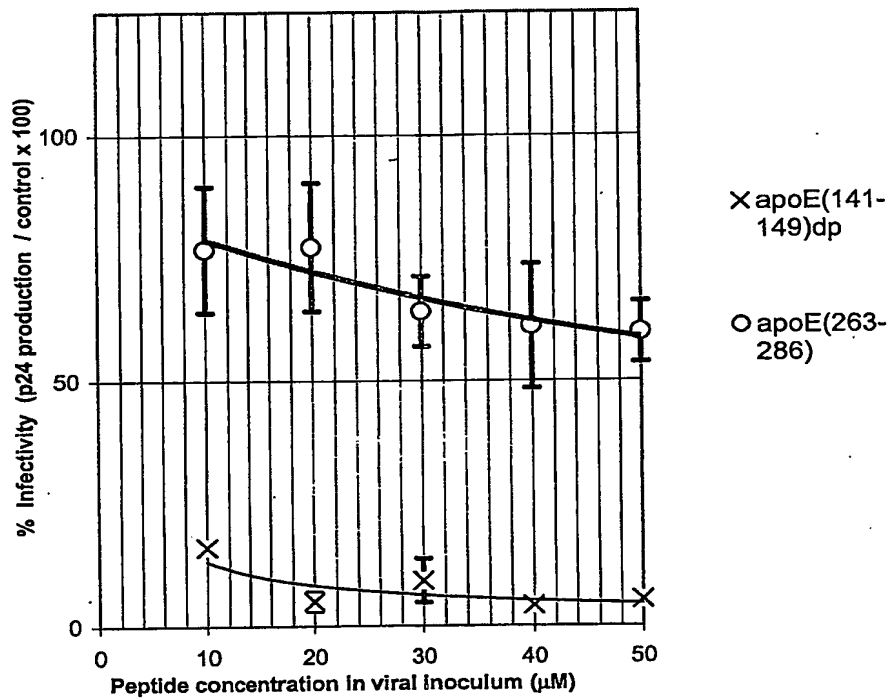


FIG. 4

Reduction in HSV1 infectivity after treatment with various concentrations of apoE derived peptides

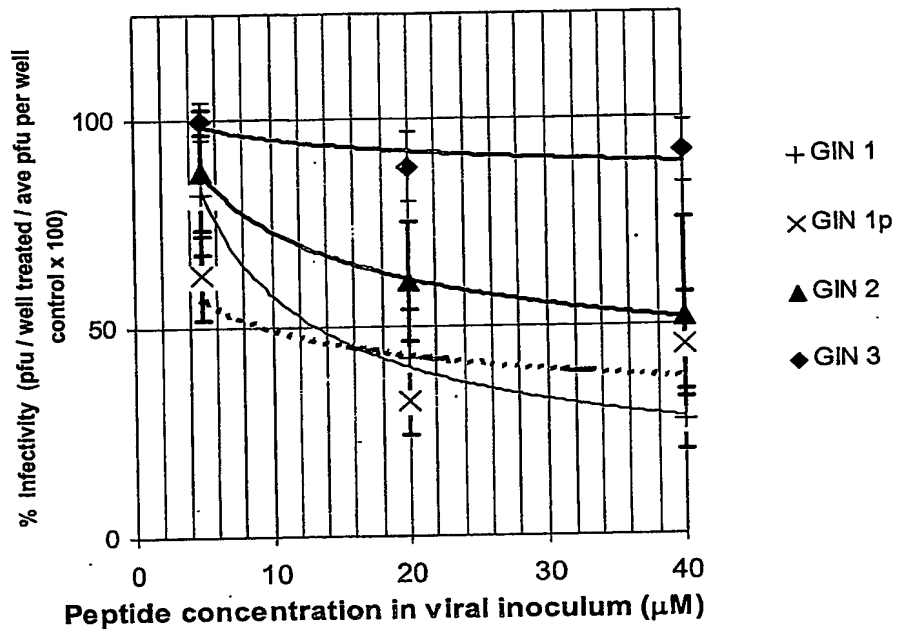


FIG. 5

Reduction in HSV1 infectivity after treatment with various concentrations of apoE derived peptides

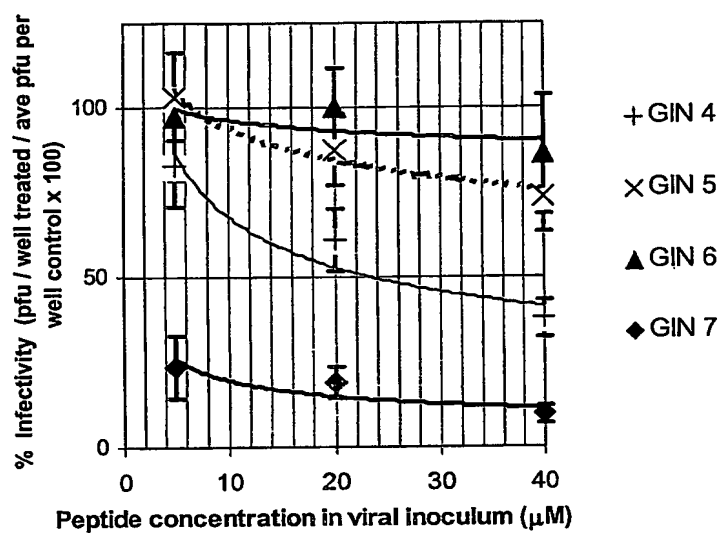


FIG. 6

Reduction in HSV1 infectivity after treatment with various concentrations
of apoE derived peptides

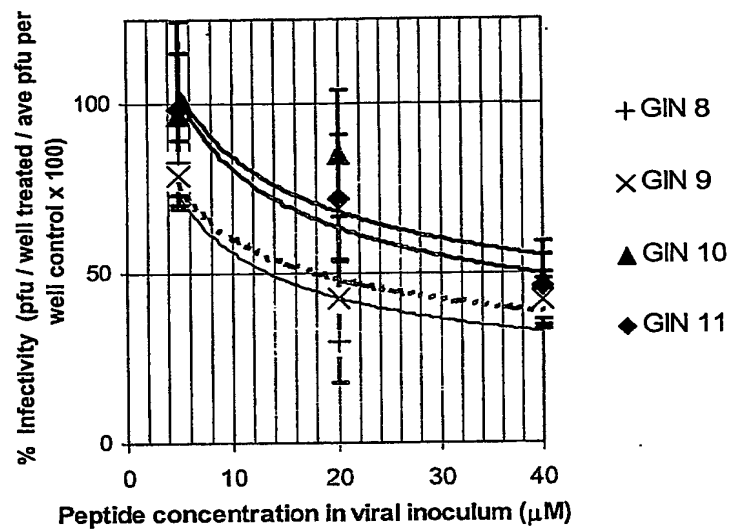


FIG. 7

Reduction in HSV1 infectivity after treatment with various concentrations of GIN peptides

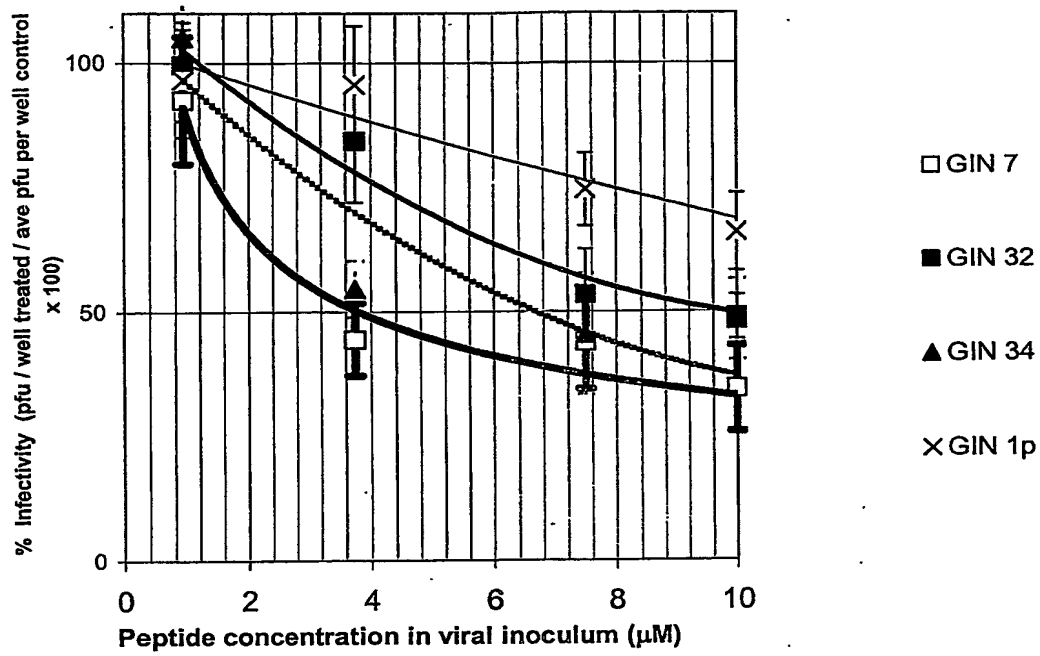
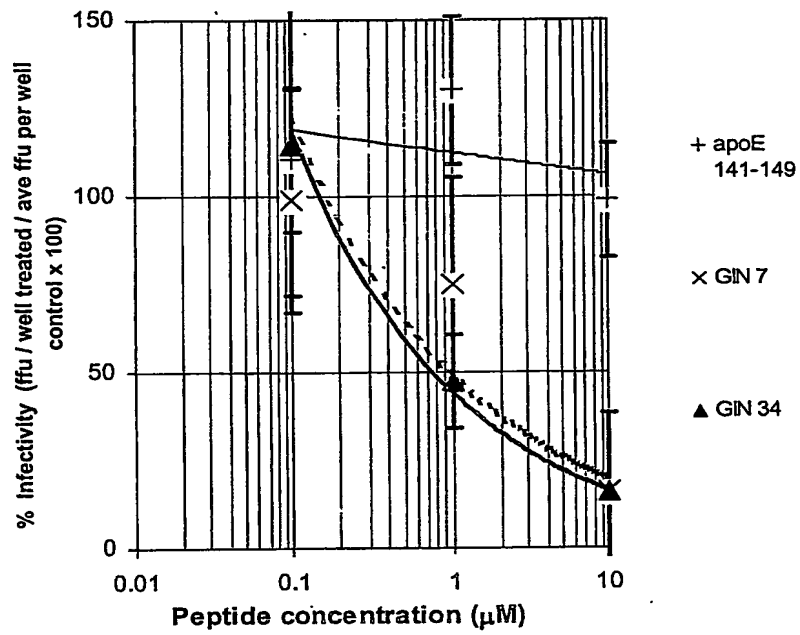


FIG. 8

Reduction in HIV infectivity after treatment of NP2 glioma cells with various concentrations of GIN peptides



Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/GB04/005360

International filing date: 17 December 2004 (17.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: GB
Number: 0329254.7
Filing date: 17 December 2003 (17.12.2003)

Date of receipt at the International Bureau: 24 January 2005 (24.01.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☒ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.